(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

REMARKS

Claims 38-76 are pending in this application. By this Amendment, Claim 38 has been amended. Claim 49 stands allowed. Claims 38-48 and 50-76 have been rejected.

In the present Office Action, Claims 38-42, 44-48, 50-62, 66-70, and 73-76 have been rejected under 35 U.S.C. 103(a) in light of the Eliason reference, the Pakula reference, the Benson reference, United States Patent No. 4,634,678 (hereinafter the "'678 patent"), and United States Patent No. 5,576,190 (hereinafter the "'190 patent"). Applicant submits that this rejection is not well taken for the following reasons.

Applicant respectfully submits that the rejected claims are inventive over the Chen reference. Applicant notes that page 5 of the Office Action states that "[i]t would have been obvious to one of ordinary skill in the art at the time of filing of the instant invention to combine the mutated DNA lambda operator sequences of Chen et al., Eliason et al., or Benson et al., with the increased thermostability of repressor sequences of Pakula et al., since Pakula et al. taught the increased thermostability of

the repressor complex was due to changes in the thermodynamic molecular interaction of specific bases and amino acids in the binding site of the operator/repressor pair."

Applicant respectfully submits that the Office Action misinterprets the Pakula reference. It is important to note that the repressor disclosed in the Pakula reference is the Cro repressor rather than the cI repressor of the present invention, which is substantially different from Cro. It should also be noted that Pakula discloses mutations in the repressor itself and not in the operator DNA sequence. An additional difference between this reference and the present invention is that the Pakula reference uses the term "thermal stability" to refer to a higher stability of the described repressor mutants towards proteolytic cleavage at a higher temperature in contrast to unstable repressor mutants. This difference is supported by the first sentence of the discussion in which it stated that "[w]e have used an antibody screen to identify randomly generated second site mutations that confer increased resistance to intracellular proteolysis upon an unstable Cro mutant." Further, in the section entitled "Mutant Screen" on page 203, it is stated that the identification of the mutants described by Pakula et al. was performed by the identification of a minimalized proteolysis at a higher temperature. As Figure 4 demonstrates, the term "thermal stability" refers to the thermal stability of the protein as distinguished from the thermal stability of the function of how repressor proteins influence the induction or repression action of a lambda promoter. Therefore, given the vast differences between this reference and the present application, Applicant submits that a person of ordinary skill in the art would not rely upon the Pakula reference when trying to obtain operator DNA mutations resulting in increased thermostability of binding to the cI repressor.

Additionally, the present invention relates to an increased thermostability of thermolabile cI repressor binding by mutation of the operator DNA sequences. The lambda cI repressor binds as a dimer to the operator. In the native lambda cI-system the repressor is proteolytically cleaved, thus losing the capability of dimerization. The present invention, however, relates to a thermolabile cI repressor mutant, e.g. cI857. In the cI857 system, raising the temperature above 30-32° C is sufficient to abolish the binding to the operator. According to the present invention, the binding of the thermolabile repressor to the mutated operator is strengthened, thus resulting in an increased thermostability. This is a completely different teaching from that of the Pakula reference.

Thus, Applicant submits that the Office Action is in error in its statements that the Pakula reference teaches the molecular mechanism of the binding of a repressor to an operator and that this makes "abundantly clear the fact that the binding of the repressor to the operator follows predictable and well known thermodynamic principles which may be affected by a mutated operator sequence." However, binding affinity results, to a certain extent, from the thermodynamic interaction of the amino acids of the repressor with the DNA base pairs of the operator. The respective amino acids which are responsible for enhanced thermal stability might occur in very different positions or might have negative effects on the affinity.

Furthermore, the last sentence of page 208 of the Pakula reference states that "[i]n fact, three of the five second site suppressor substitutions ... replace residues that have been proposed to contribute to the affinity and/or specificity of the Cro-operator DNA complex." This means that at least two of the described mutations with a thermal

stability are not due to mutations in amino acids which are responsible for DNA binding. It also states in the final paragraph of the left column of page 209 that "[t]wo of the revertant substitutions, YC26 and QL16 ... increase the thermal stability of Cro to a significant extent" and that "[t]he QL16 and QP29 substitutions reduce the apparent affinity of Cro for operator DNA by 1500- and 150-fold respectively" and "[t]he YC26 substitution, on the other hand, has only a small (2- to 8-fold) effect." Therefore, the Cro mutants with enhanced thermal stability (QL16 and YC26) have a 1500-fold (QL16 mutant) or at least an 8-fold (YC26 mutant) reduced binding affinity for the operator sequence. This is in clear contrast to the present invention. Therefore, Applicant respectfully submits that this reference actually teaches away from the present invention and that reliance upon this reference is misplaced.

The Benson reference relates to the binding of wild-type lambda repressor to mutated operator sequences, since the binding mechanism of the wild-type repressor is governed by different principles than the binding of a thermosensitive repressor (as discussed above). It was shown that a mutated operator DNA sequence can change the binding affinity of the repressor sequence. In this example, mutations have either a positive or a negative or no effect on the binding affinity of the wild-type repressor. Thus, the Benson reference itself does not show a mutant according to the present application and does not show a reliable method of obtaining mutated operator sequences having increased thermostability for the binding of a thermosensitive repressor.

The '190 patent relates to a lambda promoter system having "increased structural stability" wherein the DNA sequence located in a position 5' to the repressor

binding region has been deleted. The only mentioned operator sequence mutation leads to a "tighter regulation" (Col. 7, lines 11-20). This reference, however, fails to disclose or even discuss a different thermostability of the repressor binding.

In the previous Response, it was argued that Chen teaches mutations to the pL and pO promoter regions which employ the cI857 repressor and not mutant operator regions with an altered affinity for the cI857 repressor. However, this argument was considered unpersuasive. While it was admitted that Chen does not teach or investigate the affinity of the cI857 repressor for the mutated operator regions, the Office Action stated that the reference teaches that the pL and pO regions were mutated and that these mutations resulted in an altered expression of the reporter gene. It was further noted that while the affinity of the mutated operator sequences to the cI857 repressor was not discussed in the Chen reference, the remaining citations "nicely clarified" the issue. Applicants respectfully suggest that the above reasoning is sufficient to remove the remaining citations from consideration. However, in the event that this is insufficient to overcome the rejection, Applicants have amended Claim 38 to further differentiate it from the Chen reference.

Applicants have amended Claim 38, in particular Claim 38(b), in such a fashion that only "man-made" and not "natural" mutagenesis is encompassed by the Claims. Support for such an amendment can be found on page 5, paragraph 3 of the specification in which the process for creating mutants is detailed. Further support for such an amendment may be found on the bridging paragraph of pages 6-7 of the specification in which the variants are labeled as insertion, deletion, and substitution of bases which reads on man-made alterations. Applicant notes that Chen is restricted to

"spontaneous" or "natural" mutations and not engineered mutations and Applicant suggests that the amendments to Claim 38 differentiate the present invention from the subject matter disclosed in the Chen reference. Accordingly, given the differences between the cited prior art and the present application, which have not been overcome by the citations, Applicant respectfully requests that this rejection be withdrawn.

Claim 43 was rejected under 35 U.S.C. 103(a) as unpatentable over Chen et al. in view of Eliason et al., Pakula et al., Benson et al, US Patent No. 4,634,678 (the '678 Patent), US Patent No. 5,576,190 (the '190 Patent) and US Patent No. 5,811,093 (the '093 Patent). As Applicant has previously stated, the Pakula and Benson references and the '190 patent do not relate to the present invention. Further, the '093 reference teaches a mutator bacterial strain used for the well known mutation of a desired sequence of phage DNA. However, this reference is insufficient to compensate for the failures of the remaining references to teach the entire invention of the present application. Accordingly, Applicant respectfully requests that this rejection be withdrawn as well.

Additionally, Claims 63-65, 71 and 72 were rejected under 103(a) in view of Chen, Pakula, Benson, the '678 patent as applied to Claims 38-42, 44-48, 50-62, 66-70 and 73-76, and further in view of Szostak et al. While the Office Action admitted that the prior references do not teach the composition of vaccines and the manufacture of bacterial ghost cells with the claimed compositions through the claimed methods, the Office Action does argue that the claimed invention is, nonetheless, obvious in light of the Szostak reference. Applicant submits that because the prior arguments and amendments are sufficient to overcome the Chen, Pakula, and Benson references, this

rejection should also be withdrawn. Szostak fails to disclose, in conjunction with the remaining references, the entire invention of the present application. Accordingly, Applicant respectfully requests that this rejection be withdrawn. Therefore, Applicant respectfully submits that the application is in condition for allowance and requests that all rejections be withdrawn.

In the event this paper is not timely filed, Applicant hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,

D. Daniel Dzara, II
Registration No. 47,543

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W., Suite 600
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810

Attachments: Petition for Extension of Time
Marked Up Copy of Claims

MARKED UP COPY OF CLAIMS

(Once Amended) --38. A method for selecting OR or OL operator DNA sequences from lambdoid phages wherein said sequences have a different thermostability compared to a wild-type sequence with regard to binding a repressor, comprising

(a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,

(b) intentionally subjecting the operator DNA sequence to a non-naturally occurring mutagenesis, and

(c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.